

DNase I HYPERSENSITIVITY OF THE TERMINAL INVERTED REPEAT DNA SEQUENCES IN THE MACRONUCLEUS OF THE CILIATE *STYLONYCHIA MYTILUS*

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1. Introduction

Ciliates contain two types of nuclei: a diploid transcriptionally inactive micronucleus and a DNA-rich transcriptionally active macronucleus. After sexual reproduction of these cells the old macronucleus degenerates and a new macronucleus is formed by a micronuclear derivate. In hypotrichous ciliates (i.e., *Stylonychia*, *Oxytricha*, *Euplotes*) macronuclear development includes the formation of polytene chromosomes, elimination of >90% of the DNA and the specific fragmentation of the macronuclear genome into gene-sized DNA molecules with a size of ~0.3–20 kilobases [1–10]. All of the macronuclear DNA molecules carry the same 20 basepairs terminal inverted repeat sequence which seems to be not only identical within the same species but in all hypotrichous ciliates examined [11–14; Swanton et al., personal communication; unpublished results). This terminal sequence is 5'-C₄A₄C₄A₄C₄. These terminal sequences may be able to aggregate with each other forming multistranded DNA complexes [15]. This observation together with the high sequence conservation made it interesting to examine its organization in the chromatin of the macronucleus. We first examined the DNAase I sensitivity of this sequence and compared it to that of bulk macronuclear DNA. Since most of the macronuclear DNA molecules probably represent structural genes [5–9,16] our study deals with the DNase I sensitivity of a DNA sequence adjacent to the coding region of eucaryotic genes.

2. Material and methods

Culture of *Stylonychia mytilus* and isolation of macronuclei was performed as in [2,17]. For DNase

I-digestion macronuclei in 10 mM Tris (pH 7.5), 100 mM NaCl, 3 mM MgCl₂ were adjusted to an A₂₆₀ of 40 and 15 units DNase I (Worthington) were added per 40 A. Digestion was performed for various time intervals (see figure legends). Reaction was stopped by the addition of 10 mM EDTA and 0.5% SDS and the DNA was isolated as in [6]. Electrophoresis of macronuclear DNA was performed on 1% agarose gels [18]. DNase I sensitivity of macronuclear DNA was determined by precipitation the DNA at various time intervals after digestion with 10% perchloric acid (PCA). The precipitate was hydrolyzed in 1 N PCA for 1 h at 80°C, the DNA concentration was determined by the diphenylamine reaction.

Isolation of the terminal inverted repeat sequences of macronuclear DNA followed essentially the method in [13]. It includes 5'-phosphorylation of macronuclear DNA [19], denaturation of the DNA followed by short reannealing and subsequent digestion with single-stranded specific nuclease S1. Purity of the digestion product was determined on 10% acrylamide gels [20].

For hybridization studies 0.5–2 µg DNA isolated from DNase I-digested nuclei were loaded onto nitrocellulose filters. The amount of filter bound DNA was determined by the diphenylamine reaction. Hybridization to this DNA with the 20 basepairs terminal repeat sequence was performed for 2–4 h at 50–60°C in 4 × SSC using 0.25–0.5 µg/ml ³²P-labeled terminal repeat sequence. Following hybridization filters were extensively washed with 4 × SSC and then counted in a Packard scintillation counter.

3. Results

The DNA pattern of macronuclei digested for various time intervals with DNase I is shown in fig.1a.

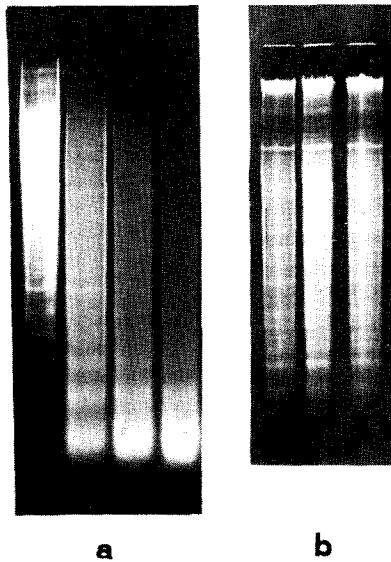


Fig.1. (a) DNA pattern of macronuclei digested with DNase I as in section 2. Samples were digested (from left to right): 0 min, 5 min, 10 min, 20 min. The % DNA remaining is under these conditions: 5 min 85%; 10 min 75%; 20 min 60–65% (b) Macronuclei were incubated under identical conditions but without DNase I. Incubation time (from left to right): 0 min, 10 min, 20 min.

Like most of the active or potentially active eucaryotic genes [21,22] macronuclear DNA sequences in total are very sensitive to DNase I-digestion. Under our digestion conditions already after 5 min 15% of macronuclear DNA is perchloric acid-soluble and after 20 min 30–40% of the DNA is digested. It is of some interest that under these conditions and in this organism clear nucleosomal bands appear on the gel. To exclude the possibility that this pattern is created by endogenous nucleases we incubated macronuclei under identical conditions but without DNase I. As shown in fig.1b there is little if any endogenous nuclease activity under these conditions.

The DNA of DNase I-digested macronuclei (fig.1a) was loaded onto nitrocellulose filters and the ^{32}P -labeled terminal repeat sequence was hybridized to them. Due to the low sequence complexity of this sequence [13,15] hybridization occurs very fast. Thus we did not observe significant differences in the hybridization to the different DNA preparations whether we hybridized for 2 h or 4 h. The result of this hybridization experiment is shown in fig.2a,b. There is a dramatic decrease in hybridization to the terminal repeat sequence already after very short digestion with

DNase I. After a 5 min digest >50% of the terminal inverted repeat sequences are lost while only ~15% of the bulk DNA is digested. After 20 min digestion 30–40% of total macronuclear DNA sequences are PCA-soluble but already 80% of the terminal sequences are digested.

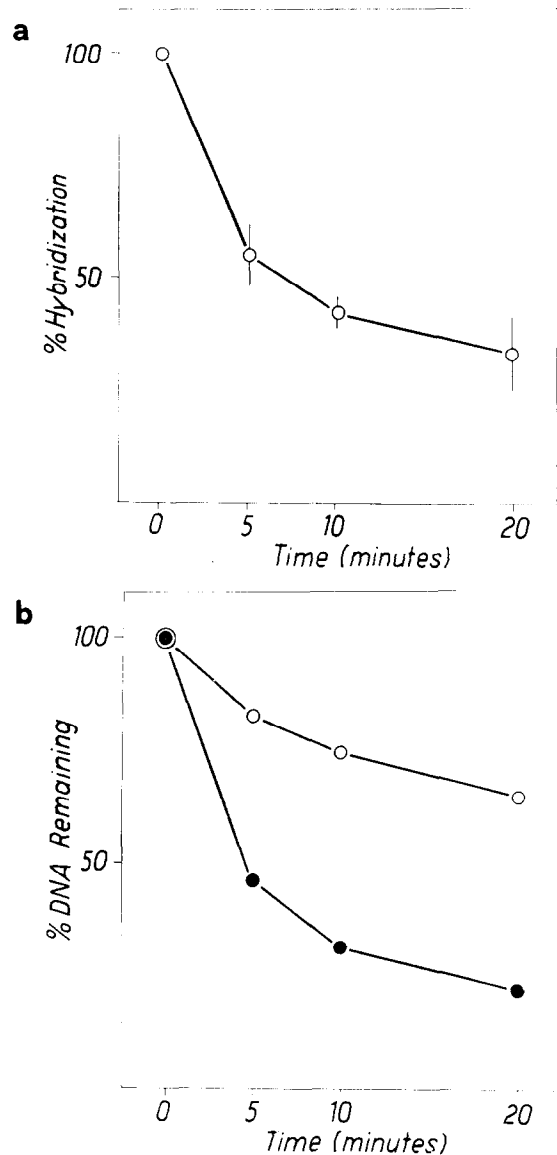


Fig.2. Hybridization of the 20 basepairs terminal inverted repeat sequence to the DNA of DNase I-digested (0–20 min) macronuclei. (a) DNA (2 μg) was loaded onto nitrocellulose filters; 0.25–0.5 $\mu\text{g}/\text{ml}$ ^{32}P -labeled terminal repeat sequence were hybridized to these filters in 4 \times SSC at 55°C for 4 h. (b) Comparison of the DNase I sensitivity of bulk macronuclear DNA (\circ) with that of the terminal inverted repeat sequence (\bullet).

4. Discussion

This report deals with the DNase I sensitivity of a specific DNA sequence adjacent to the coding regions of structural genes. Hypotrichous ciliates are a particularly good system for these kind of experiments since the DNA of the macronucleus is already in vivo fragmented into gene-sized DNA molecules. Since $\geq 60\%$ of macronuclear DNA sequences are transcribable into nuclear RNA [16], most of the DNA molecules represent at least potentially active genes. It therefore has to be assumed that the gene-sized DNA pieces should carry all DNA sequences necessary for its transcription and replication. We choose a defined DNA sequence present on all macronuclear DNA molecules since its high sequence conservation throughout evolution and its ability to form multistranded DNA complexes with each other suggests that it is important for the cell.

The DNase I sensitivity of active genes is now widely found [21,22]. In agreement with this finding total macronuclear DNA sequences are very sensitive to DNase I when compared for example to micronuclear DNA sequences (not shown). Control experiments clearly showed that the DNA pattern observed after DNase I-digestion is not due to the presence of endogenous nucleases.

In viral chromatin [23–25] and in some eucaryotic genes like the heat-shock genes of *Drosophila* [26,27], chicken globin gene [28] and *Drosophila* histone genes (cited in [29]) there exist DNA sequences adjacent to the coding region of the genes which are hypersensitive to DNase I-digestion. Such sequences may be important in the regulation of transcription of eucaryotic genes [27]. So far, the nucleotide sequence of these regions has not been determined and it is not clear whether they are related to each other. Our results also show that a sequence closed to the coding region of the genes is preferentially digested by DNase I, suggesting to be in another chromatin configuration than bulk DNA sequences. This sequence is very simple, furthermore it is present on all macronuclear genes of *Stylonychia*. With these methods we cannot decide whether the terminal sequences present on both sides of the molecule are equally sensitive to DNase I or whether there exists a polarity in the chromatin structure of the molecules. However, using the advantages of the ciliate macronucleus, the relative ease in isolating large amounts of a defined sequence, it should be possible to make a more detailed analysis of its organiza-

tion in chromatin and to analyze the proteins associated to it.

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